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Bioactivities of extracts from *Acorus gramineus* on four stored grain pests

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Abstract: Botanical insecticides are relatively safe and biodegradable, and readily available source of bioinsecticides. In recent years, the studies on new species of plant and on the spectrum of them have been much heated in the world. In this study, four different extracts (ethanol extract, acetone extract, ethyl acetate extract and petroleum ether extract) of *Acorus gramineus* rhizome were tested for their insecticidal activities against four stored grain pests, *Sitophilus zeamais* Motschusky, *Rhizopertha dominica* Fabricius, *Cryptolestes pusillus* Schoenherr and *Oryzaephilus surinamensis* L., using repellence method and contact toxicity method. The results showed that all extracts had obviously repellent and contact toxicity on the tested beetles. Sixty hours after the treatment, the repellent levels of the four extracts to the insects were all above grade Ⅲ. The contact mortalities of these four tested insects ranged from 41.11% to 100.00%. The results suggest that *A*. *gramineus* extracts can be used to control the main stored grain pests effectively, and offer basis for developing *A*. *gramineus* as the plant insecticide to control the stored grain pests.

Key words: *Acorus gramineus*; extract; *Sitophilus zeamais*; *Rhizopertha dominica*; *Cryptolestes pusillus*; *Oryzaephilus surinamensis*; repellent toxicity; contact toxicity

1 INTRODUCTION

Control of the stored product insects around the world is primarily dependent upon insecticides such as organophosphates and pyrethroids, and gaseous insecticides such as methyl bromide and phosphine. Although effective, their repeated use for decades has disrupted biological control by natural enemies and led to outbreak of insect species, development of resistance to the synthetic chemical, undesirable effects on non-target organisms, and environmental and human health concerns (Champ and Dyte, 1976; Rajendran and Narasimhan, 1994; Subramanyam and Hagstrum, 1995; White and Leesch, 1995). These problems have highlighted the need to develop new type of stored grain insecticides.

Many plant extracts and essential oils may be alternative source of stored grain insect-control agents because they constitute rich sources of bioactive chemicals. Much effort has, therefore, been focused on plant-derived materials as potential sources of commercial insect-control agents or as lead compounds (Yao *et al*., 2004, 2005).

Acorus gramineus belongs to the family Phizoma arisaematis, the rhizome from *A*. *gramineus* has long been considered to have medicinal properties such as digestant, expectorant, or stimulant against digestive disorders, diarrhoea and epilepsy. It contains various compounds such as (Z)-asarone (63% - 81%), (E)-asarone (8% - 14%), caryophyllene (1% - 4%), isoasarone (0.8% - 3.4%), (Z)-methyl isoeugenol (0.3% - 6.8%) and safrol (0.1% - 1.2%) (Tang and Eisenbrand, 1992; Namba, 1993). However, little work has been done to consider its potential to manage stored product insects. But more recently the insecticidal activities of a methanol extract of the *Acorus* rhizome against *Mythimna separata*, *S*. *oryzae* and *C*. *chinensis* as well as adults of *Nilaparvata lugens* Stål and larvae of *P*. *xylostella* have been reported (Lee *et al*., 2002; Park *et al*., 2003; Xie *et al*., 2004).

In this paper we reported the repellent and contact toxicity of *A*. *gramineus* rhizome extracts from four different solvents (i. e., ethanol, acetone, ethyl acetate and petroleum ether) against four stored grain pests, *Sitophilus zeamais* Motschusky (Coleoptera: Curculionidae), *Rhizopertha dominica* Fabricius (Coleoptera: Bostrychidae), *Cryptolestes pusillus* Schoenherr (Coleoptera: Cucujidae) and *Oryzaephilus surinamensis* L. (Coleoptera: Silvanidae), with the expectation to offer basis for developing a plant insecticide to control stored grain pests.

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2 MATERIALS AND METHODS

2.1 Plant material

Rhizomes of *A*. *gramineus* were purchased from Tongrentang Medical Herb Shop in Wuhan.

2.2 Preparation of plant extracts

A. *gramineus* rhizomes were grounded into fine powder using a blender, which was sieved (40 mesh) to get uniform pore size. The powder was extracted twice with ethanol, acetone, ethyl acetate and petroleum ether (boiling between 60℃ – 90℃) at room temperature respectively and filtered (Whatman No.2). All chemicals were reagent grade. The combined filtrate was concentrated to dryness by rotating evaporation. Then the extracts were kept in refrigerator at 4℃ before the experiment. Based on the weight of the dried rhizome, the yield of an extractant is calculated with the following formula: yield (%) = dry weight of extract/dry weight of test plant × 100. The yields of ethanol, acetone, ethyl acetate and petroleum ether were 8.91%, 6.32%, 6.21% and 3.71%, respectively in this study.

2.3 Insects

Cultures of *S*. *zeamais*, *R*. *dominica*, *C*. *pusillus* and *O*. *surinamensis* were maintained in the laboratory without exposure to any insecticide. *S*. *zeamais* and *R*. *dominica* were reared on wheat while *C*. *pusillus* and *O*. *surinamensis* on wheat flour mixed with 5% yeast (w∶w) at (27 ± 1) ℃, 75% ± 5% RH and 16L∶8D photoperiod. In tests 21 day-old adults were used.

2.4 Bioassay

2.4.1 Repellency bioassay: The solution (0.13 g/mL) of each extract was used. A circular filter paper (9 cm diameter, Whatman No. 2) was cut into two equal parts, one was treated with 0.5 mL diluted extract, producing coverage of 1.97 mg/cm^2, and the other was treated with 0.5 mL acetone as control; both were then dried under a fume hood for 2 min. The two halved filter paper were then spliced again, attached to the undersurface of Petri dish (9 cm diameter), which was covered with a glass smeared with polytetraflu-oroethylene on the inside wall of the dish in order to avoid tested insects creeping off. Thirty unsexed adults were placed in each dish. Insect repellence within the Petri dishes was examined at 12, 24, 36, 48 and 60 h after treatment. All experiments were performed under ambient laboratory condition as described in section 2.3 above. All treatments were repeated three times. A repellent rate was calculated with the formula: Repellent rate (%) = $(C - T)/(C + T) \times 100$, where C is the numbers of insects present on the control areas of the discs and T is the numbers of insects present on the treated side. Based on the repellent rate, the effect of the repellency was assigned to repellency classes from 0 to Ⅴ: class 0, without any repellency; class Ⅰ, 0.1% – 20%; class Ⅱ, 20.1% – 40%; class Ⅲ, 40.1% – 60%; class Ⅳ, 60.1% – 80%; class Ⅴ, 80.1% – 100% (Malik and Muiutaba, 1984).

2.4.2 Contact toxicity: A 1 mL amount of solution (0.13 g/mL) of each extract was applied to a filter paper (9 cm diameter, Whatman No. 2). This procedure produced 1.97 mg/cm^2 dosage. Controls were treated with 1 mL acetone. Dried under a fume hood for 2 min, each filter paper was then placed in a Petri dish (9 cm diameter). Thirty unsexed adults were placed in each treated Petri dish, which was covered with a glass cup smeared with polytetraflu-oroethylene in order to avoid tested insects creeping off. The set up were maintained under the conditions given in section.

2.4.3 Cumulative mortality was recorded four days after treatment. All treatments were replicated three times. Insects were presumed dead if they did not move when touched with a brush.

2.5 Statistical analysis

The percentage mortality was determined and transformed to arcsine square-root values for analysis of variance (ANOVA). Means of treatments were compared and separated by Scheffe's test at $P = 0.05$ and $P = 0.01$ (SAS, 1990). Means (± *SE*) of untransformed data were reported.

3 RESULTS

3.1 Repellency

Repellent toxicity of *A*. *gramineus* extracts against *S*. *zeamais*, *R*. *dominica*, *C*. *pusillus* and *O*. *surinamensis* were observed in the test (Table 1). Responses varied according to insects and plant material. At the dose of 1.97 mg/cm^2, the repellent action of four different extracts to *R*. *dominica*, *C*. *pusillus* and *O*. *surinamensis* was better than that to *S*. *zeamais*. At 60 h after treatment, the repellent action of four different dissolvent extracts to *R*. *dominica*, *C*. *pusillus*, and *O*. *surinamensis* were all up to class Ⅴ, while to *S*. *zeamais* only class Ⅲ-class Ⅳ. The repellent rates of four different dissolvent extracts to *R*. *dominica*, *C*. *pusillus* and *O*. *surinamensis* were all more than 94.01%, significantly higher than that to *S*. *zeamais* ($P < 0.01$).

Repellent toxicity varied among different dissolvents. The repellent toxicities of acetone extract and ethyl acetate extract to *S*. *zeamais* were obviously stronger than that of ethanol extract, and there was no obvious difference between petroleum ether extract and other three dissolvent extracts. No obviously different action among four different dissolvent extracts was observed in the test of *R*. *dominica*, *C*. *pusillus* and

O. *surinamensis*：the repellent rate to *R*. *dominica* was up to 96.05%，and the lowest in average repellent rate to *C*. *pusillus* was also up to 99.65%.

Table 1　Repellent rate of *Acorus gramineus* extracts to four stored grain pests

Extract	Insect	Repellent rate (%)						Level
		12 h	24 h	36 h	48 h	60 h	Mean ± *SE*	
Ethanol extract	*S*. *zeamais*	81.79	83.08	61.11	19.85	-29.17	43.33 ± 1.76 e C	Ⅲ
	R. *dominica*	94.54	100.00	100.00	100.00	100.00	98.91 ± 0.64 ab AB	Ⅴ
	C. *pusillus*	100.00	100.00	100.00	98.25	100.00	99.65 ± 0.35 ab AB	Ⅴ
	O. *surinamensis*	95.34	100.00	98.15	100.00	91.40	96.98 ± 0.87 bc AB	Ⅴ
Acetone extract	*S*. *zeamais*	91.34	95.15	83.33	61.47	-15.02	63.26 ± 2.27 d C	Ⅳ
	R. *dominica*	98.25	100.00	98.25	100.00	93.75	98.05 ± 1.95 ab AB	Ⅴ
	C. *pusillus*	100.00	100.00	100.00	100.00	100.00	100.00 ± 0.00 a A	Ⅴ
	O. *surinamensis*	94.54	96.49	94.54	86.34	98.15	94.01 ± 0.35 c B	Ⅴ
Ethyl acetate extract	*S*. *zeamais*	82.18	53.89	81.41	50.84	36.21	60.91 ± 1.33 d C	Ⅳ
	R. *dominica*	98.25	96.30	100.00	98.25	96.39	97.84 ± 1.27 abc AB	Ⅴ
	C. *pusillus*	100.00	100.00	100.00	100.00	100.00	100.00 ± 0.00 a A	Ⅴ
	O. *surinamensis*	98.25	98.25	96.30	96.49	96.19	97.09 ± 1.33 abc AB	Ⅴ
Petroleum ether extract	*S*. *zeamais*	92.77	80.06	49.88	20.24	16.67	51.92 ± 4.84 de C	Ⅲ
	R. *dominica*	100.00	92.13	100.00	100.00	88.10	96.05 ± 2.04 abc AB	Ⅴ
	C. *pusillus*	100.00	100.00	100.00	100.00	100.00	100.00 ± 0.00 a A	Ⅴ
	O. *surinamensis*	100.00	98.25	100.00	96.49	98.25	98.60 ± 0.93 abc AB	Ⅴ

Each datum represents the mean of three replicates(± *SE*)，each sets up with 30 adults ($n = 90$). The data within a column followed by the same capital letters are not significantly different at $P = 0.01$ (Scheffe 's test). The data within a column followed the same small letters are not different at $P = 0.05$ (Scheffe 's test). The same for Table 2.

3.2　Contact action

The toxicity of *A*. *gramineus* extracts against the four insects with direct contact was recorded in Table 2. Significant differences in toxicity of the extracts among the insects were observed. The contact action of the extracts to *R*. *dominica*，*C*. *pusillus* and *O*. *surinamensis* was better than that to *S*. *zeamais* ($P < 0.01$). There was no obvious difference among the contact action of the extracts to *R*. *dominica*，*C*. *pusillus* and *O*. *surinamensis* ($P > 0.05$). Four days after treatment，the mortalities were all above 98.33%，but the mortalities of *S*. *zeamais* were only 41.11% - 48.89%. No significant difference in the contact action was observed among the four different dissolvent extracts to the same insect ($P > 0.05$).

Table 2　Contact activity of *Acorus gramineus* extracts to four stored grain pests

Insect	Mortality(%)			
	Ethanol extract	Acetone extract	Ethyl acetate extract	Petroleum ether extract
S. *zeamais*	47.19 ± 5.62 b B	41.11 ± 4.84 b B	45.56 ± 2.94 b B	48.89 ± 2.23 b B
R. *dominica*	100.00 ± 0.00 a A	100.00 ± 0.00 a A	98.33 ± 1.67 a A	98.33 ± 1.67 a A
C. *pusillus*	100.00 ± 0.00 a A	100.00 ± 0.00 a A	100.00 ± 0.00 a A	100.00 ± 0.00 a A
O. *surinamensis*	100.00 ± 0.00 a A	100.00 ± 0.00 a A	100.00 ± 0.00 a A	100.00 ± 0.00 a A

Each datum represents the mean of three replicates(± *SE*)，each sets up with 30 adults ($n = 90$). Insects were exposed for four days.

4　DISCUSSION

In former study，the insecticidal constituents of *A*. *gramineus* rhizome were identified as the phenylpropenes (Z)- and (E)-asarones with species selective activity. (Z)-asarone was much more effective against adults of *S*. *oryzae*，*L*. *serricorne* and *C*. *chinensis* in sealed containers than in open ones. These results suggested that the insecticidal mode of action of (Z)-asarone might be largely attributable to fumigant action，it may be toxic by penetrating the insect body via the respiratory system (Park *et al*.，2003).

Four insects in this study all belong to Coleoptera，while the repellent and contact action of *A*. *gramineus* extracts against *S*. *zeamais* were not as good as that to other three insects. This may be attributed to the different action mechanism of extracts among the insects，which needs further study.

The polarities of the four dissolvents were different：they were declining in the order of ethanol，acetone，ethyl acetate and ethyl acetate. Therefore，the materials extracted by them were different，and what are the main active ingredients of the extracts need further study.

The insects studied in this test were all major stored grain pests. Results of this study and earlier studies indicated that *A*. *gramineus* extracts could be useful for managing populations of the stored product insects in such enclosed spaces as storage bins， glasshouses or buildings.

Works are in progress for investigating insecticidal constituents against coleopterus stored product insects from extracts of *A. gramineus*， insecticidal mode of action of the constituents and appropriate formulation types in our laboratory.

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石菖蒲提取物对四种储粮害虫的生物活性研究

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摘要：研究了石菖蒲4种溶剂无水乙醇、丙酮、乙酸乙酯、石油醚提取物对玉米象、谷蠹、长角扁谷盗和锯谷盗4种储粮害虫的驱避作用和触杀作用。结果表明 石菖蒲4种溶剂提取物对4种试虫均有明显的驱避作用和触杀作用，处理60 h的平均驱避等级均达到Ⅲ级以上，对4种试虫的触杀死亡率均达到41.11%以上。

关键词：石菖蒲；提取物；玉米象；谷蠹；长角扁谷盗；锯谷盗；触杀作用；驱避作用

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